昆虫学报 *Acta Entomologica Sinica*, March 2008, 51(3):342－348 ISSN 0454-6296

# Molecular cloning and sequence analysis of chitinase cDNA from the cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

PIAO Dong-Hua, YAO Lei, WANG Ling, FAN Dong*

(Agronomy College, Northeast Agricultural University, Harbin 150030, China)

**Abstract**: Insect chitinases have been considered as potential insecticidal molecules for development of insect control strategies. Total RNA was isolated from the midgut of the cutworm, *Agrotis ipsilon* (Hufnagel) prepupae. The cDNA sequence was cloned by rapid amplification of cDNA ends (RACE). The cDNA, 2 823 base pairs in length, contained an open reading frame of 1 674 base pairs coding a polypeptide of 558 amino acid residues with a predicted molecular weight of 62.5 kDa and pI 5.12. The deduced amino acid sequence contained two putative N-glycosylation sites and twenty putative O-glycosylation sites, and two conserved domains in chitinases, *i.e.* the highly conserved catalytic domain at the N-terminus and the chitin-binding type 2 domain at the C-terminus. The predicted protein shared extensive similarities with chitinases from other insects, especially with the lepidopteran insects. The sequence has been deposited with GenBank under accession no. EU035316.

**Key words**: *Agrotis ipsilon* (Hufnagel); chitinase; cloning; sequence analysis

## 1 INTRODUCTION

Chitinases (EC 3.2.1.14) have been found in a variety of organisms, including bacteria, fungi, plants, marine organisms, insects, and mammals (Choi *et al.*, 1997; Gemma and David, 1995; Fukamizo, 2000). Insect chitinases play roles in various physiological processes, such as hatching, moulting, nutrient acquisition and defence mechanisms against pathogens (Flach *et al.*, 1992). In insects, chitin is the major component of the cuticle and the peritrophic membranes. Chitinases are responsible for chitin metabolism (Cohen-Kupiec and Chet, 1998). To date, several insect chitinase cDNA sequences have been identified and published from the *Manduca sexta*, *Bombyx mori*, *Hyphantria cunea*, *Spodoptera litura*, *Choristoneura fumiferana*, *Helicoverpa armigera*, *Lacanobia oleracea*, *Spodoptera frugiperda*, *Phaedon cochleariae*, *Tenebrio molitor*, *Chelonus* sp. and *Locusta migratoria manilensis* and other insects (Kramer *et al.*, 1993; Krishnan *et al.*, 1994; Kim *et al.*, 1998; Girard and Jouanin, 1999; Goo *et al.*, 1999; Shinoda *et al.*, 2001; Royer *et al.*, 2002; Ahmad *et al.*, 2003; Fitches *et al.*, 2004; Bolognesi *et al.*, 2005; Genta *et al.*, 2006; Zhang *et al.*, 2007).

Insect chitinases identified so far belong to the family 18 of glycosyl hydrolases that include the chitinases from arthropods, bacteria, fungi, viruses, and class III chitinases from plants (Henrissat, 1991). Insect chitinases are secreted proteins and commonly contain a catalytic domain, a chitin-binding domain 2 (6-Cys domain) and a linker, named Pro/Glu/Ser/Thr-rich (PEST) region, between catalytic domain and chitin-binding domain 2 (Kramer and Muthukrishnan, 1997; Arakane *et al.*, 2003). All the lepidopteran chitinases show the basic structure which consists of three domains.

In insects, chitinases are induced stage- and tissue- dependently to degrade the chitin in the exoskeleton and peritrophic membrane (Kramer *et al.*, 1993; Kramer and Muthukrishnan, 1997). With the unique and important functions, insect chitinases have been considered as potential insecticidal molecules for development of insect control strategies (Kramer and Muthukrishnan, 1997). Several insect chitinase genes have been engineered into transgenic plants to enhance their insect tolerance and into microbial entomopathogens to increase their insect control efficacies (Wang *et al.*, 1996; Kramer and Muthukrishnan, 1997; Ding *et al.*, 1998). The recombinant baculoviruses or *Bacillus thuringiensis* or plant expressing insect chitinase could perturb

---

基金项目：黑龙江省自然科学基金项目(C2007-7)；黑龙江省教育厅科学技术研究项目(11521020)

作者简介 朴冬花，女，1984年9月生，硕士研究生，主要从事昆虫生物化学与分子生物学研究，E-mail：donghua820112@sina.com

*通讯作者 Author for correspondence, Tel.：0451-55190539；Fax：0451-55190447；E-mail：dnfd@163.com

收稿日期 Received：2007-10-21；接受日期 Accepted：2008-01-03

peritrophic membrane function of infected insects, leading to higher susceptibility to other harmful compounds (Merzendorfer and Zimoch, 2003).

We report here the cDNA cloning and sequence analysis of a chitinase from the cutworm, *Agrotis ipsilon* (Hufnagel) [= *Agrotis ypsilon* (Rottemberg)]. An understanding of the chitinase in this pest will facilitate the development of chitinase-based insecticides for the biological control.

## 2 MATERIALS AND METHODS

### 2.1 Experimental insects

The culture of *A*. *ipsilon* was maintained in the laboratory under controlled conditions of temperature 25℃, 70% relative humidity, and a photoperiod of 12 h light:12 h dark. The larvae were reared on corn leaf diet until prepupation.

### 2.2 RNA extraction and isolation of the full-length cDNA of *A*. *ipsilon* chitinase by rapid amplification of cDNA ends (RACE)

Total RNA was prepared from the midgut of *A*. *ipsilon* prepupae using Trizol (r) reagent (Invitrogen) according to the method provided by the supplier and stored at −86℃ until used. RNA integrity was verified by running an aliquot of the RNA sample on 1% denaturing agarose gel stained with ethidium bromide (EtBr).

5 μg of total RNA from *A*. *ipsilon* mixed with oligo(dT) adaptor primer 5′- GAC TCG AGT CGA CAT CGA(dT)17-3′ was incubated at 70℃ for 5 min and then placed on ice. The RNA-oligo(dT) adaptor primer hybrid mixture was then mixed in a 20 μL reaction containing 20 U/μL superscript® Reverse Transcriptase Ⅱ (Invitrogen), 2 U/μL of RNasin ribonuclease inhibitor (TaKaRa), Reverse Transcriptase buffer and 0.25 mmol/L dNTP (TaKaRa). The reverse transcriptase reaction was carried out at 42℃ for 50 min followed by 50℃ for 20 mim. 1 μL of the above cDNA was used for amplification by PCR with the sense primer 5′-TTT GAT GGA TTG GAC CTG GAC TGG GAG T-3′ and the antisense primer 5′-TCC ATG TCA ATA GCC CAG GTC AAG GCT CC-3′ located in the known conservative regions of lepidopteran chitinase cDNAs of *M*. *sexta* (Kramer *et al*., 1993), *B*. *mori* (Kim *et al*., 1998), *H*. *cunea* (Kim *et al*., 1998), *S*. *litura* (Shinoda *et al*., 2001) and *H*. *armigera*. (Ahmad *et al*., 2003). The obtained PCR products were cloned into a pGEM-T Easy Vector (Promega, WI, USA), and transformed into JM109 chemically competent cells. Following plasmid DNA purification, the sequence of each construct was determined using an automated sequencer by TAKARA company and was used to design the 5′- and 3′-RACE specific primers.

Rapid amplification of cDNA ends (RACE) was carried out using a BD SMART™ RACE cDNA Amplification Kit (Clontech Laboratories, CA, USA). Reverse transcription and adapter ligation were performed following the manufacture's protocol. The 5′ end of the cDNA was PCR amplified in an amplification reaction (50 μL) containing 2.5 μL of 5′ RACE-Ready cDNA, 0.2 mmol/L deoxyribonucleotides, 10 μmol/L of a gene-specific primer GSPR1 (5′-CAG AAC ATC TTG ATC TCC ACA CTG CGA-3′), 10 μmol/L of the Universal Primer Mix supplied in the kit, and 2 units of ExTaq DNA polymerase (TaKaRa, Japan). Temperature cycling was carried out at 94℃ for 3 min, followed by five cycles of 30 s at 94℃ and 3 min at 72℃, five cycles of 30 s at 94℃, 30 s at 70℃, and 3 min at 72℃, and 20 cycles of 30 s at 94℃, 30 s at 68℃, and 3 min at 72℃. The 3′ end of the cDNA was PCR-amplified in an amplification reaction (50 μL) containing 2.5 μL of 3′ RACE-Ready cDNA, 0.2 mmol/L deoxyribonucleotides, 10 μmol/L of a gene-specific primer GSPF1 (5′- GCC TTG ACC TGG GCT ATT GAC ATG-3′), 10 μmol/L of the adaptor primer 5′- GAC TCG AGT CGA CAT CGA-3′, and 2 units of ExTaq DNA polymerase (TaKaRa, Japan). Temperature cycling was carried out under the same conditions as for 5′-RACE.

The obtained 5′- and 3′-RACE PCR products were cloned into a pGEM-T Easy Vector, transformed into JM109 chemically competent cells and sequenced. The resulting overlapping sequences were assembled to obtain the full-length chitinase cDNA sequence.

### 2.3 DNA and protein sequence analyses

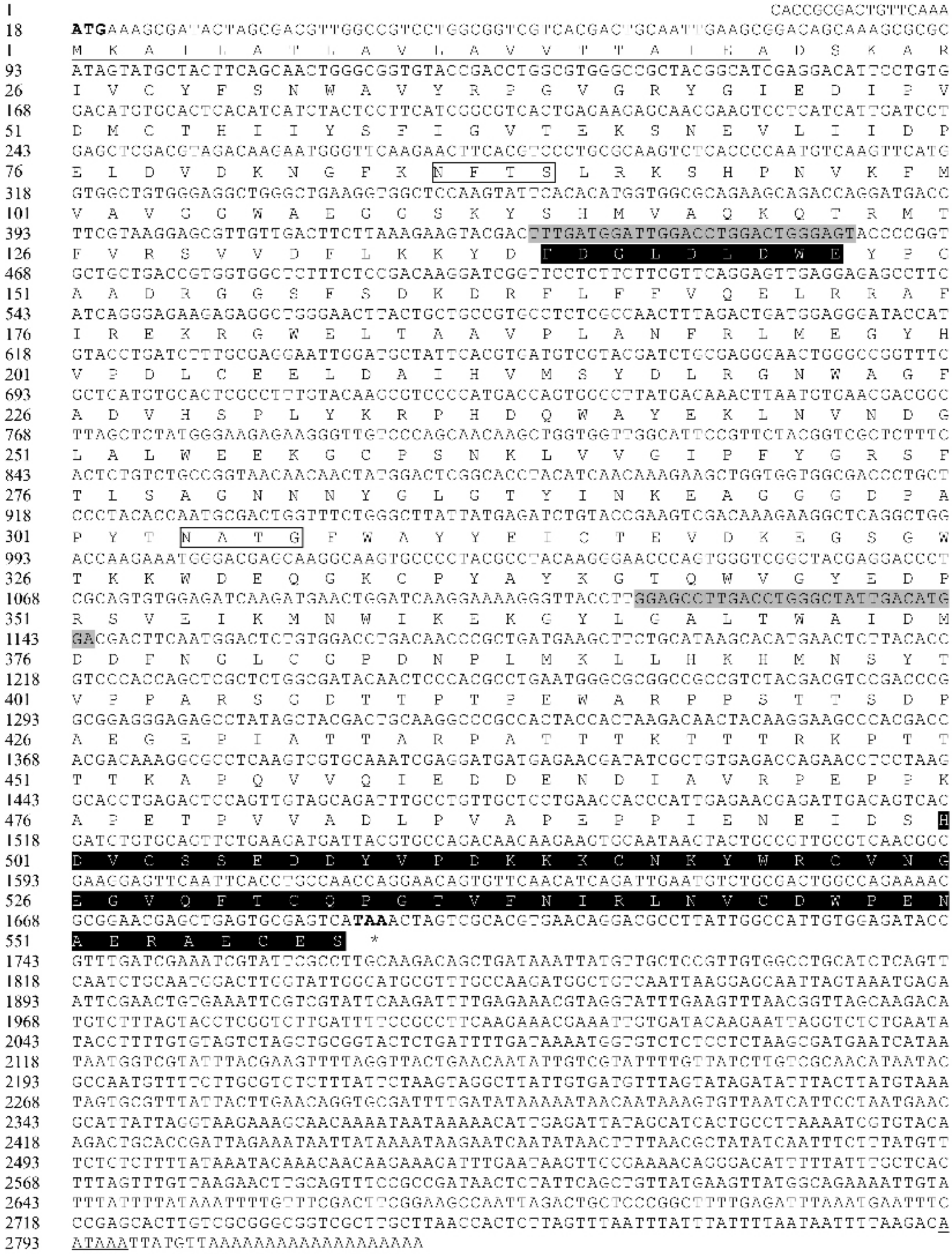
The amino acid sequence of chitinase was deduced following translation of the corresponding cDNA sequence using the translation tool at the ExPASy Proteomics website (http://us.expasy.org/tools/ dna.html). Other protein sequence analysis tools used in this study, including MW, pI, and conserved domains prediction, were obtained from the ExPASy Proteomics website (http://us.expasy.org/). Multiple sequence alignments of nucleotide and deduced amino acid sequences were made using multiple alignment software ClustalW. The phylogenetic tree constructed with DNAMAN program. The signal peptide was determined by SignalPv3.1. Prediction of putative PEST sites and *N*- and *O*-glycosylation sites was performed with the programs PESTfind (https://emb1.bcc.univie.ac.at/ toolbox/ pestfind/ pestfind-analysis-webtool.htm), PROSCAN (http://npsa-pbil.ibcp.fr/cgi-bin/ npsa-automat.pl?page=npsa_prosite.html) and NetOGlyc 3.1 (http://www.cbs.dtu.dk/services/NetOGlyc/), respectively (Rogers *et al*., 1986; Bairoch *et al*., 1997；Hansen *et al*.，1997）.

# 3 RESULTS

## 3.1 Sequences analysis of chitinase cDNA and deduced amino acid

The obtained 5′- and 3′-RACE PCR products were about 1 100 and 2 400 base pairs，respectively. After sequencing，overlapping sequences were assembled to obtain the full-length chitinase cDNA sequence.

The *A*. *ipsilon* chitinase cDNA was 2 823 bp in length and contained an open reading frame of 1 674 bp flanked by a 5′ untranslated region of 17 bp and a 3′ untranslated region of 1 132 bp. A putative polyadenylation signal sequence AATAAA was located



```
1                                                               CACCGCGACTGTTCAAA
18    ATGAAAGCGATACTAGCGACGTTGGCCGTCCTGGCGGTCGTCACGACTGCAATTGAAGCGGACAGCAAAGCGCGC
1      M  K  A  I  L  A  T  L  A  V  L  A  V  V  T  T  A  I  E  A  D  S  K  A  R
93    ATAGTATGCTACTTCAGCAACTGGGCGGTGTACCGACCTGGCGTGGGCCGCTACGGCATCGAGGACATTCCTGTG
26     I  V  C  Y  F  S  N  W  A  V  Y  R  P  G  V  G  R  Y  G  I  E  D  I  P  V
168   GACATGTGCACTCACATCATCTACTCCTTCATCGGCGTCACTGACAAGAGCAACGAAGTCCTCATCATTGATCCT
51     D  M  C  T  H  I  I  Y  S  F  I  G  V  T  D  K  S  N  E  V  L  I  I  D  P
243   GAGCTCGACGTAGACAAGAATGGGTTCAAGAACTTCACGTCCCTGCGCAAGTCTCACCCCAATGTCAAGTTCATG
76     E  L  D  V  D  K  N  G  F  K  N  F  T  S  L  R  K  S  H  P  N  V  K  F  M
318   GTGGCTGTGGGAGGCTGGGCTGAAGGTGGCTCCAAGTATTCACACATGGTGGCGCAGAAGCAGACCAGGATGACC
101    V  A  V  G  G  W  A  E  G  G  S  K  Y  S  H  M  V  A  Q  K  Q  T  R  M  T
393   TTCGTAAGGAGCGTTGTTGACTTCTTAAAGAAGTACGACTTTGATGGATTGGACCTGGACTGGGAGTACCCCGGT
126    F  V  R  S  V  V  D  F  L  K  K  Y  D  F  D  G  L  D  L  D  W  E  Y  P  G
468   GCTGCTGACCGTGGTGGCTCTTTCTCCGACAAGGATCGGTTCCTCTTCTTCGTTCAGGAGTTGAGGAGAGCCTTC
151    A  A  D  R  G  G  S  F  S  D  K  D  R  F  L  F  F  V  Q  E  L  R  R  A  F
543   ATCAGGGAGAAGAGAGGCTGGGAACTTACTGCTGCCGTGCCTCTCGCCAACTTTAGACTGATGGAGGGATACCAT
176    I  R  E  K  R  G  W  E  L  T  A  A  V  P  L  A  N  F  R  L  M  E  G  Y  H
618   GTACCTGATCTTTGCGAGGAATTGGATGCTATTCACGTCATGTCGTACGATCTGCGAGGCAACTGGGCCGGTTTC
201    V  P  D  L  C  E  E  L  D  A  I  H  V  M  S  Y  D  L  R  G  N  W  A  G  F
693   GCTGATGTGCACTCGCCTTTGTACAAGCGTCCCCATGACCAGTGGGCCTATGAGAAACTTAATGTCAACGACGGC
226    A  D  V  H  S  P  L  Y  K  R  P  H  D  Q  W  A  Y  E  K  L  N  V  N  D  G
768   TTAGCTCTATGGGAAGAGAAGGGTTGTCCCAGCAACAAGCTGGTGGTTGGCATTCCGTTCTACGGTCGCTCTTTC
251    L  A  L  W  E  E  K  G  C  P  S  N  K  L  V  V  G  I  P  F  Y  G  R  S  F
843   ACTCTGTCTGCCGGTAACAACAACTATGGACTCGGCACCTACATCAACAAAGAAGCTGGTGGTGGCGACCCTGCT
276    T  L  S  A  G  N  N  N  Y  G  L  G  T  Y  I  N  K  E  A  G  G  G  D  P  A
918   CCCTACACCAATGCGACTGGTTTCTGGGCTTATTATGAGATCTGTACCGAAGTCGACAAAGAAGGCTCAGGCTGG
301    P  Y  T  N  A  T  G  F  W  A  Y  Y  E  I  C  T  E  V  D  K  E  G  S  G  W
993   ACCAAGAAATGGGACGAGCAAGGCAAGTGCCCCTACGCCTACAAGGGAACCCAGTGGGTCGGCTACGAGGACCCT
326    T  K  K  W  D  E  Q  G  K  C  P  Y  A  Y  K  G  T  Q  W  V  G  Y  E  D  P
1068  CGCAGTGTGGAGATCAAGATGAACTGGATCAAGGAAAAGGGTTACCTTGGAGCCTTGACCTGGGCTATTGACATG
351    R  S  V  E  I  K  M  N  W  I  K  E  K  G  Y  L  G  A  L  T  W  A  I  D  M
1143  GACGACTTCAATGGACTCTGTGGACCTGACAACCCGCTGATGAAGCTTCTGCATAAGCACATGAACTCTTACACC
376    D  D  F  N  G  L  C  G  P  D  N  P  L  M  K  L  L  H  K  H  M  N  S  Y  T
1218  GTCCCACCAGCTCGCTCTGGCGATACAACTCCCACGCCTGAATGGGCGCGGCCGCCGTCTACGACGTCCGACCCG
401    V  P  P  A  R  S  G  D  T  T  P  T  P  E  W  A  R  P  P  S  T  T  S  D  P
1293  GCGGAGGGAGAGCCTATAGCTACGACTGCAAGGCCCGCCACTACCACTAAGACAACTACAAGGAAGCCCACGACC
426    A  E  G  E  P  I  A  T  T  A  R  P  A  T  T  T  K  T  T  T  R  K  P  T  T
1368  ACGACAAAGGCGCCTCAAGTCGTGCAAATCGAGGATGATGAGAACGATATCGCTGTGAGACCAGAACCTCCTAAG
451    T  T  K  A  P  Q  V  V  Q  I  E  D  D  E  N  D  I  A  V  R  P  E  P  P  K
1443  GCACCTGAGACTCCAGTTGTAGCAGATTTGCCTGTTGCTCCTGAACCACCCATTGAGAACGAGATTGACAGTCAC
476    A  P  E  T  P  V  V  A  D  L  P  V  A  P  E  P  P  I  E  N  E  I  D  S  H
1518  GATGTGTGCAGTTCTGAAGATGATTACGTGCCAGACAAGAAGAAGTGCAATAAGTACTGGCGTTGCGTCAACGGC
501    D  V  C  S  S  E  D  D  Y  V  P  D  K  K  K  C  N  K  Y  W  R  C  V  N  G
1593  GAAGGAGTTCAATTCACCTGCCAACCAGGAACAGTGTTCAACATCAGATTGAATGTCTGCGACTGGCCAGAAAAC
526    E  G  V  Q  F  T  C  Q  P  G  T  V  F  N  I  R  L  N  V  C  D  W  P  E  N
1668  GCGGAACGAGCTGAGTGCGAGTCATAAACTAGTCGCACGTGAACAGGACGCCTTATTGGCCATTGTGGAGATACC
551    A  E  R  A  E  C  E  S  *
1743  GTTTGATCGAAATCGTATTCGCCTTGCAAGACAGCTGATAAATTATGTTGCTCCGTTGTGGCCTGCATCTCAGTT
1818  CAATCTGCAATCGACTTCGTATTGGCATGCGTTTGCCAAGATGGCTGTCAATTAAGGAGCAATTAGTAAATGAGA
1893  ATTCGAACTGTGAAATTCGTCGTATTCAAGATTTTGAGAAACGTAGGTATTTGAAGTTTAACGGTTAGCAAGACA
1968  TGTCTTTAGTACCTCGGTCTTGATTTTCCGCCTTCAAGAAACGAAATTGTGATACAAGAATTAGGTCTCTGAATA
2043  TACCTTTTGTGTAGTCTAGCTGCGGTACTCTGATTTTGATAAAATGGTGTCTCTCCTCTAAGCGATGAATCATAA
2118  TAATGGTCGTATTTACGAAGTTTTAGGTTACTGAACAATATTGTCGTATTTTGTTATCTTGTCGCAACATAATAC
2193  GCCAATGTTTTCTTGCGTCTCTTTATTCTAAGTAGGCTTATTGTGATGTTTAGTATAGATATTTACTTATGTAAA
2268  TAGTGCGTTTATTACTTGAACAGGTGCGATTTTGATATAAAAATAACAATAAAGTGTTAATCATTCCTAATGAAC
2343  GCATTATTAGGTAAGAAAGCAACAAAATAATAAAAACATTGAGATTATAGCATCACTGCCTTAAAATCGTGTACA
2418  AGACTGCACCGATTAGAAATAATTATAAAATAAGAATCAATATAACTTTTAACGCTATATCAATTTCTTTATGTT
2493  TCTCTCTTTTATAAATACAAACAACAAGAAACATTTGAATAAGTTCCCGAAAACAGGGACATTTTTATTTGCTCAC
2568  TTTAGTTTGTTAAGAACTTGCAGTTTCCGCCGATAACTCTATTCAGCTGTTATGAAGTTATGGCAGAAAATTGTA
2643  TTTATTTTATAAATTTTGTTTCGACTTCGGAAGCCAATTAGACTGCTCCCGGCTTTTGAGATTTAAATGAATTTC
2718  CCGAGCACTTGTCGCGGGCGGTCGCTTGCTTAACCACTCTTAGTTTAATTTATTTATTTTAATAATTTTAAGACA
2793  ATAAATTATGTTAAAAAAAAAAAAAAAAAAA
```

Fig. 1　Nucleotide and deduced amino acid sequences of *Agrotis ipsilon* chitinase

The initial codon ATG and termination codon TAA are indicated in bold，and the putative signal peptide of the deduced amino acid sequence is underlined. Two putative N-glycosylation sites are boxed. Chitinases family 18 active site FDGLDLDWE and the conserved chitin-binding type 2 domain are shaded solid black. The cDNA amplification primers are shaded grey. A putative polyadenylation site（AATAAA）is double-underlined. The numbers on the left are for nucleotide sequence and amino acid sequence. The GenBank accession number for this sequence is EU035316.

at 13 bp upstream of the polyA tail（Fig. 1）. The ORF encoded a polypeptide of 558 amino acid residues with a predicted molecular weight of 62.5 kDa and a pI of 5.12. With the Signal P V3.1 program，a hydrophobic signal peptide of 20 residues was predicted at the N-terminus of the protein. Prosite and PROSCAN programs' scanning of the protein sequence revealed a number of putative posttranslational modification sites and the highly conserved Chitinases family 18 active site FDGLDLDWE from amino acid residue 139 to 147 and the conserved chitin-binding type 2 domain with six conserved cysteines from amino acid residue 500 to 558. Two putative *N*-glycosylation sites were identified at the positions 86 – 89 and 304 – 307. The sequence of *A*. *ipsilon* chitinase was well conserved at the catalytic and the chitin binding domains and other features highly similar to those identified from other insects，especially to lepidopterans（Table 1）. These features indicated that the protein was a member of family 18 glycosyl hydrolases. The sequence has been deposited with GenBank with accession no. EU035316 under the species name *Agrotis ypsilon*.

**Table 1　Characteristics of some chitinases from different insect orders and their amino acid identities with *Agrotis ipsilon***

| Insect | GenBank accession no. | Mw | pI | PEST sites | N-glycosylation sites | O-glycosylation site number | Identities with *A*. *ipsilon*（%） |
|---|---|---|---|---|---|---|---|
| *Agrotis ipsilon* | EU035316 | 62.5 | 5.12 | 405-417，417-436，475-500 | 86 – 89 NFTS；304-307 NATG. | 20 | — |
| *Mythimna sepatata* | AY508698 | 62.8 | 5.05 | 417 – 436，481 – 500 | 86 – 89 NFTD；304 – 307 NATG | 21 | 513/559（91） |
| *Spodoptera exigua* | AY678531 | 62.6 | 5.20 | 417 – 436，474 – 499 | 86 – 89 NFTA；304 – 307 NATG | 23 | 502/557（90） |
| *S*. *frugiperda* | AY527414 | 62.3 | 5.29 | 417 – 436，472 – 497 | 86 – 89 NFTA；304 – 307 NATG | 19 | 500/557（89） |
| *S*. *litura* 1 | AB032107 | 62.2 | 5.16 | 417 – 433，469 – 494 | 86 – 89 NFTA；304 – 307 NATG | 16 | 497/557（89） |
| *S*. *litura* 2 | AY325497 | 62.4 | 5.26 | 417 – 433，469 – 494 | 86 – 89 NFTA；304 – 307 NATG | 16 | 496/557（89） |
| *Lacanobia*. *oleracea* | AJ620505 | 61.8 | 5.18 | 417 – 436，473 – 495 | 86 – 89 NFTA；304 – 307 NATG | 17 | 496/561（88） |
| *Helicoverpa armigera* | AY325496 | 65.7 | 6.02 | 417 – 435，493 – 520 | 86 – 89 NFTA；304 – 307 NATG | 29 | 494/578（85） |
| *Hyphantria cunea* | U86877 | 62.0 | 4.96 | 417 – 444，444 – 458，486 – 508 | 86 – 89 NFTA；304 – 307 NATG | 18 | 465/556（83） |
| *Bombyx mori* 3 | AF273695 | 61.1 | 5.01 | 418 – 441，472 – 500 | 87 – 90 NFTS；305 – 308 NATG | 26 | 452/558（81） |
| *B*. *mori* 1 | NM-001044015 | 61.0 | 5.13 | 417 – 440，471 – 499 | 86 – 89 NFTS；304 – 307 NATG；398 – 401 NYTV | 27 | 450/556（80） |
| *B*. *mori* 2 | AB052914 | 61.0 | 5.17 | 417 – 440，471 – 499 | 86 – 89 NFTS；304 – 307 NATG | 27 | 449/557（80） |
| *B*. *mori* 4 | AF455139 | 63.5 | 5.15 | 418 – 441，472 – 500 | 87 – 90 NFTS；305 – 308 NATG | 28 | 451/557（80） |
| *Chilo suppressalis* | AY705930 | 61.4 | 5.17 | 416 – 439，456 – 507 | 65 – 68 NTSE；85 – 88 NFTA；303 – 306 NATG | 29 | 449/561（80） |
| *Manduca sexta* | U02270 | 62.2 | 5.32 | 404 – 416，416 – 437，474 – 508 | 85 – 88 NFTS；303 – 306 NATG；545 – 548 NATR | 24 | 438/542（80） |
| *B*. *mandarina* | AF326596 | 63.5 | 5.21 | 417 – 440，471 – 499 | 86 – 89 NFTS；304 – 307 NATG；398 – 401 NYTV | 27 | 443/556（79） |
| *Ostrinia furnacalis* 2 | DQ294305 | 61.9 | 5.28 | 418 – 439，460 – 497 | 87 – 90 NFTS；305 – 308 NATG | 30 | 433/545（79） |
| *O*. *furnacalis* 1 | AY726548 | 61.8 | 5.28 | 417 – 438，459 – 496 | 86 – 89 NFTS；304 – 307 NATG | 29 | 442/561（78） |
| *Choristoneura fumiferana* | AY098731 | 62.2 | 5.39 | 417 – 437，473 – 499 | 86 – 89 NFTS；304 – 307 NATG | 18 | 435/561（77） |
| *O*. *furnacalis* 3 | DQ887770 | 61.9 | 5.28 | 357 – 379，400 – 437 | 87 – 90 NFTS；305 – 308 NATG；359 – 362 NWST | 24 | 431/555（77） |
| *Tribolium castaneum* | NM_001039435 | 60.1 | 5.80 | 414 – 425，447 – 476 | 84 – 87 NFTN；302 – 305 NATG | 22 | 347/555（62） |
| *Aedes aegypti* | AF026491 | 64.3 | 4.83 | 394 – 408，413 – 436，451 – 471 | 83 – 86 NFTN；301 – 304 NASG | 28 | 341/559（61） |
| *Nasonia vitripennis* | XM_001602730 | 61.0 | 6.08 | 423 – 439，466 – 481 | 70 – 73 NVTW | 18 | 341/558（61） |
| *Drosophila pseudoobscura* | XM_001358387 | 61.5 | 6.48 | 409 – 430，470 – 482，496 – 512 | 70 – 73 NVTW | 29 | 330/556（59） |
| *D*. *melanogaster* | NM_142057 | 67.1 | 6.14 | 419 – 441，483 – 496，496 – 534 | 307 – 310 NASG | 31 | 337/579（58） |
| *Apis mellifera* | XM_397146 | 56.1 | 6.55 | 387 – 460 | 214 – 217 NGSW；274 – 277 NSTN；415 – 418 NTTI；464 – 467 NCSI | 37 | 165/401（41） |
| *Locusta migratoria manilensis* | EF090723 | 51.5 | 4.83 | 394 – 437 | – | 30 | 155/379（40） |
| *Chelonus* sp | CSU10422 | 52.0 | 5.41 | – | – | 2 | 204/558（36） |
| *Tenebrio molitor* | AJ487081 | 321.4 | 6.49 | 50 – 65，1 047 – 1 072，1 275 – 1 291<br>1 438 – 1 453，1 454 – 1 470<br>2 347 – 2 368，2 415 – 2 462 | 162 – 165 NVSQ；459 – 462 NETQ；806 – 809 NFTK；889 – 892 NYTM；1 574 – 1 577 NDSQ；1 715 – 1 718 NFSI；2 005 – 2 008 NDSA；2 146 – 2 149 NFTI；2 542 – 2 545 NDSA；2 682 – 2 685 NFSL | 85 | 192/547（35） |
| *A*. *gambiae* | XM_316448 | 52.4 | 5.07 | 352 – 403，403 – 430 | 212 – 215 NASI；402 – 405 NHTT | 31 | 191/548（34） |

Mw：Molecular weight（kDa）deduced from cDNA；pI：Isoelectric point.

### 3.2 Homology and phylogenetic analysis with other insect chitinases

All the nineteen chitinase amino acid sequences from lepidopteran insects and another ten chitinase amino acid sequences from other orders of insects were used to make homology comparison and phylogenetic analysis with A. ipsilon chitinase. Sequences from lepidopteran insects included U02270 (*M. sexta*), NM_001044015 (*B. mori*), AB052914 (*B. mori*), AF2736953 (*B. mori*), AF455139 (*B. mori*), U86877 (*H. cunea*), AB032107 (*S. litura*), AY325497 (*S. litura*), AY678531 (*Spodoptera exigua*), AY508698 (*Mythimna separata*), AY726548 (*Ostrinia furnacalis*), DQ294305 (*Ostrinia furnacalis*), DQ887770 (*Ostrinia furnacalis*), AY705930 (*Chilo suppressalis*), AY098731 (*C. fumiferana*), AY325496 (*H. armigera*), AJ620505 (*L. oleracea*), AY527414 (*S. frugiperda*), and AF326596 (*B. mandarina*). Sequence from non-lepidopteran insects were NM_001039435 (*Tribolium castaneum*), AF026491 (*A. aegypti*), XM_001602730 (*Nasonia vitripennis*), XM_001358387 (*Drosophila pseudoobscura*), NM_142057 (*D. melanogaste*), XM_397146 (*Apis mellifera*),

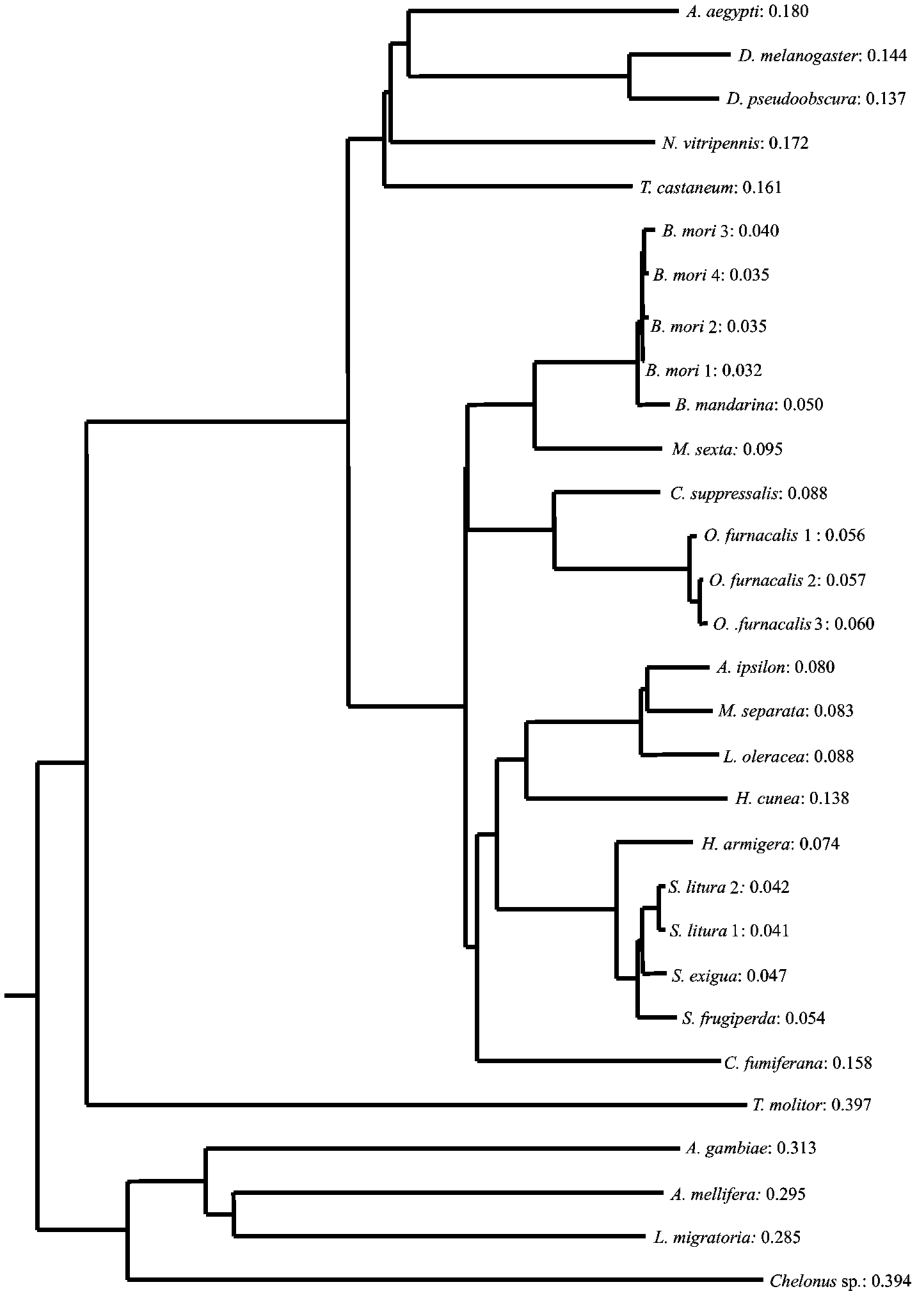


Fig. 2 Phylogenetic tree of insect chitinase amino acid sequences

The lines at left indicate the relationship between insects chitinase. The numbers on the right are sequence weights.

EF090723（*L*. *migratoria manilensis*），CSU10422（*Chelonus* sp.），AJ487081（*T*. *molitor*）and XM_316448（*Anopheles gambiae*）. The alignment was conducted with all the twenty chitinase amino acid sequences from lepidopteran insects including *A*. *ipsilon*.

The homology comparison and alignment of the deduced amino acid sequence from *A*. *ipsilon* with those from other insects suggested that *A*. *ipsilon* chitinase was strikingly similar to those from insects of Lepidoptera（over 77% in amino acid identity），especially to the chitinases from other Noctuidae of Lepidoptera（over 85% in amino acid identity）（Table 1）. The protein sequence shared an amino acid identity of 91% to the chitinase from *M*. *separata*，90% to the chitinase from *S*. *exigua*，and 85% to the chitinase from *H*. *armigera*. The sequence of the *A*. *ipsilon* chitinase was less similar to the chitinases from non-lepidopteran species（below 62% in amino acid identity）（Table 1）. The protein sequence shared an amino acid identity of 61% to the chitinase from *A*. *aegypti*，36% to the chitinase from *Chelonus* sp.，and 35% to the chitinase from *T*. *molitor*.

The phylogenetic tree（Fig. 2）indicated a phylogenetic relationship between *A*. *ipsilon* chitinase and other insect chitinases. The amino acid sequence of *A*. *ipsilon* chitinase was closely related to those from lepidopteran insects，especially to those from Noctuidae except for *H*. *cunea* which belonged to the family of Arctiidae but it was a member of Noctuidea. The amino acid sequence of *A*. *ipsilon* chitinase was distantly related to those from insect species in other families of lepidopteran and other non-lepidopteran insects，such as *M*. *sexta*，*B*. *mori* and *T*. *castaneum*（Fig. 2）.

## 4 DISCUSSION

In insects，chitinases are important in the gut for degradation of PM to allow for efficient absorption of nutrients，and they also play an important role in ecdysis for cuticle degradation during development（Yan *et al*.，2002）. Insect chitinases have been characterized from larva guts and cuticle. This is the insect chitinase to be reported expressed in midgut.

In this paper，a chitinase cDNA was cloned from the cutworm，*A*. *ipsilon*. Sequence analysis indicated that the *A*. *ipsilon* chitinase sequence was highly similar to chitinase amino acid sequences from other insect species，especially those in the order of Lepidoptera.

A hydrophobic signal peptide of 20 residues predicted at the N-terminus of the protein indicated that the *A*. *ipsilon* chitinase was a secreted protein. *A*. *ipsilon* chitinase amino acid sequence also showed the basic structure which consisted of three domains. This chitinase protein contained conserved chitinases family 18 active site FDGLDLDWE from amino acid residue 139 to 147，the conserved chitin-binding type 2 domain with six conserved cysteines from amino acid residue 500 to 558 and a PEST linker with three PEST sites between catalytic domain and chitin-binding domain 2. These features suggested that this sequence belonged to the family of 18 glycosyl hydrolases.

## References

Ahmad T，Rajagopal R，Bhatnagar RK，2003. Molecular characterization of chitinase from polyphagous pest *Helicoverpa armigera*. *Biochem*. *Biophys*. *Res*. *Commun*.，310（1）：188－195.

Arakane Y，Zhu QS，Matsumiy M，Muthukrishnan S，Kramer KJ，2003. Properties of catalytic，linker and chitin-binding domains of insect chitinase. *Insect Biochem*. *Mol*. *Biol*.，33：631－648.

Bairoch A，Bucher P，Hofmann K，1997. The PROSITE database，its status in 1997. *Nucleic Acids Res*.，25：217－221

Bolognesi R，Arakane Y，Muthukrishnan S，Kramer KJ，Terra WR，Ferreira C，2005. Sequences of cDNAs and expression of genes encoding chitin synthase and chitinase in the midgut of *Spodoptera frugiperda*. *Insect Biochem*. *Mol*. *Biol*.，35（11）：1 249－1 259.

Choi HK，Choi KH，Kramer KJ，Muthukrishnan S，1997. Isolation and characterization of a genomic clone for the gene of an insect molting enzyme，chitinase. *Insect Biochem*. *Mo*. *Biol*.，27（1）：37－47.

Cohen-Kupiec R，Chet I，1998. The molecular biology of chitin digestion. *Curr*. *Opin*. *Biotechnol*.，9：270－277.

Ding X，Gopalakrishnan B，Johnson LB，White FF，Wang X，Morgan TD，Kramer KJ，Muthukrishnan S，1998. Insect resistance of transgenic tobacco expressing an insect chitinase gene. *Transgenic Res*.，7：77－84.

Fitches E，Wilkinson H，Bell H，Bown DP，Gatehouse JA，Edwards JP，2004. Cloning，expression and functional characterisation of chitinase from larvae of tomato moth（*Lacanobia oleracea*）：a demonstration of the insecticidal activity of insect chitinase. *Insect Biochem*. *Mol*. *Biol*.，34（10）：1 037－1 050

Flach J，Pilet PE，Jolles P，1992. What's new in chitinase research？*Experientia*，48：701－716.

Fukamizo T，2000. Chitinolytic enzymes：catalysis，substrate binding and their applications. *Curr*. *Protein Pep*. *Sci*.，1：105－124.

Gemma ME，David JA，1995. Chitinase activity in human serum and leukocytes. *Infection and Immunity*，63（12）：4 770－4 773.

Genta FA，Blanes L，Cristofoletti PT，Lago CL，Terra WR，Ferreira C，2006. Purification，characterization and molecular cloning of the major chitinase from *Tenebrio molitor* larval midgut. *Insect Biochem*. *Mol*. *Biol*.，36（10）：789－800.

Girard C，Jouanin L，1999. Molecular cloning of a gut-specific chitinase cDNA from the beetle *Phaedon cochleariae*. *Insect Biochem*. *Mol*. *Biol*.，29（6）：549－556.

Goo TW，Hwang JS，Sung GB，Yun EY，Bang HS，Kwon OY，1999. Molecular cloning and characterization of the gene encoding chitinase from *Bombyx mandarina*. *J*. *Life Sci*.，9（4）：341－347.

Merzendorfer H，Zimoch L，2003. Chitin metabolism in insects：structure，function and regulation of chitin synthases and chitinases. *J*. *Exp*. *Biol*.，206：4 393－4 412.

Hansen JE，Lund O，Rapacki K，Brunak S，1997. OGLYCBASE version 2.0：a revised database of O-glycosylated proteins. *Nucleic Acids Res*.，25：278－282.

Henrissat B，1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem*. *J*.，280：309－316.

Kim MG，Shin SW，Bae KS，Kim SC，Park HY，1998. Molecular cloning of chitinase cDNAs from the silkworm，*Bombyx mori* and the fall webworm，*Hyphantria cunea*. *Insect Biochem*. *Mol*. *Biol*.，28（3）：163－171.

Kramer KJ, Corpuz L, Choi HK, Muthukrishnan S, 1993. Sequence of a cDNA and expression of the gene encoding epidermal and gut chitinases of *Manduca Sexta*. *Insect Biochem. Mol. Biol.*, 23(6): 691 - 701.

Kramer KJ, Muthukrishnan S, 1997. Insect chitinases: molecular biology and potential use as biopesticides. *Insect Biochem. Mol. Biol.*, 27(11): 887 - 900.

Krishnan A, Nair PN , Jones D, 1994. Isolation, cloning, and characterization of new chitinase stored in active form in chitin-lined venom reservoir. *J. Biol. Chem.*, 269(33): 20 971 - 20 976.

Rogers S, Wells R, Rechsteiner M, 1986. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science*, 234: 364 - 368.

Royer V, Fraichard S , Bouhin H, 2002. A novel putative insect chitinase with multiple catalytic domains: hormonal regulation during metamorphosis. *Biochem J.*, 366: 921 - 928.

Shinoda T, Kobayashi J, Matsui M, Chinzei Y, 2001. Cloning and functional expression of a chitinase cDNA from the common cutworm, *Spodoptera litura*, using a recombinant baculovirus lacking the virus-encoded chitinase gene. *Insect Biochem. Mol. Biol.*, 31(6 - 7): 521 - 532.

Wang XR, Ding XF, Gopalakrishnan B, Kramer KJ, 1996. Characterization of a 46 kDa insect chitinase from transgenic tobacco. *Insect Biochem. Mol. Biol.*, 36(10): 1 055 - 1 064.

Yan J, Cheng Q, Narashimhan S, Li CB, Aksoy S, 2002. Cloning and functional expression of a fat body-specific chitinase cDNA from the tsetse fly, *Glossina morsitans morsitans*. *Insect Biochem. Mol. Biol.*, 32: 979 - 989.

Zhang BS, Cao YQ, Zhang W, Yin YP, Wang ZK, Peng GX, Xia YX, 2007. Cloning, sequence analysis and tissue expression of chitinase gene from the midgut of *Locusta migratoria manilensis* (Meyen). *Acta Entomologica Sinica*, 50(6): 555 - 559. [**张八生,曹月青,张伟,殷幼平,王中康,彭国雄,夏玉先**,2007. **东亚飞蝗中肠几丁质酶基因的克隆、序列分析及组织定位. 昆虫学报**,50(6): 555 - 559]

**(责任编辑:袁德成)**

---

# 小地老虎几丁质酶基因的克隆与序列分析

**朴冬花,姚 磊,王 玲,樊 东***

**(东北农业大学农学院,哈尔滨** 150030)

**摘要:利用昆虫几丁质酶对几丁质的调控作用破坏几丁质新陈代谢的平衡来防治害虫,在生物防治策略中具有很大的发展潜力。从处于预蛹期的小地老虎** *Agrotls ipsilon* (Hufnagel)**体中肠内提取总的** RNA,**经反转录,利用** cDNA **末端快速扩增技术**(RACE)**获得了几丁质酶基因的** cDNA **序列。该基因序列已经登录** GenBank **并获得登录号为** EU035316。**该序列长度为** 2 823 **个碱基,含有一个** 1 674 **个碱基的开放读码框。开放读码框编码** 558 **个氨基酸残基,预测的分子量为** 62.5 kDa,**等电点** 5.12。**推导得到的氨基酸序列含有** 2 **个** N-**位糖基化位点,**20 **个** O-**位糖基化位点,含有** 2 **个几丁质酶所具有的保守序列:**N-**端的催化区和** C-**端的几丁质结合区。氨基酸序列与其他昆虫,特别是鳞翅目昆虫的几丁质酶高度同源。**

**关键词:小地老虎;几丁质酶;克隆;序列分析**

**中图分类号:**Q966 **文献标识码:**A **文章编号:**0454-6296(2008)02-0342-07